GENETIC VARIATION IN NATURAL POPULATIONS: DNA

The number of genes prescribing a eukaryotic life form such as a Douglas fir or human being runs into the tens of thousands. The nucleotide pairs composing them vary among species from one billion to ten billion. If the DNA helices in one cell of a mouse, a typical animal species, were placed end on end and magically enlarged to have the same width as wrapping string, they would extend for over nine hundred kilometers, with about four thousand nucleotide pairs packed into very meter. Measured in bits of pure information, the genome of a cell is comparable to all editions of the Encyclopedia Britannica *published since its inception in 1768.*

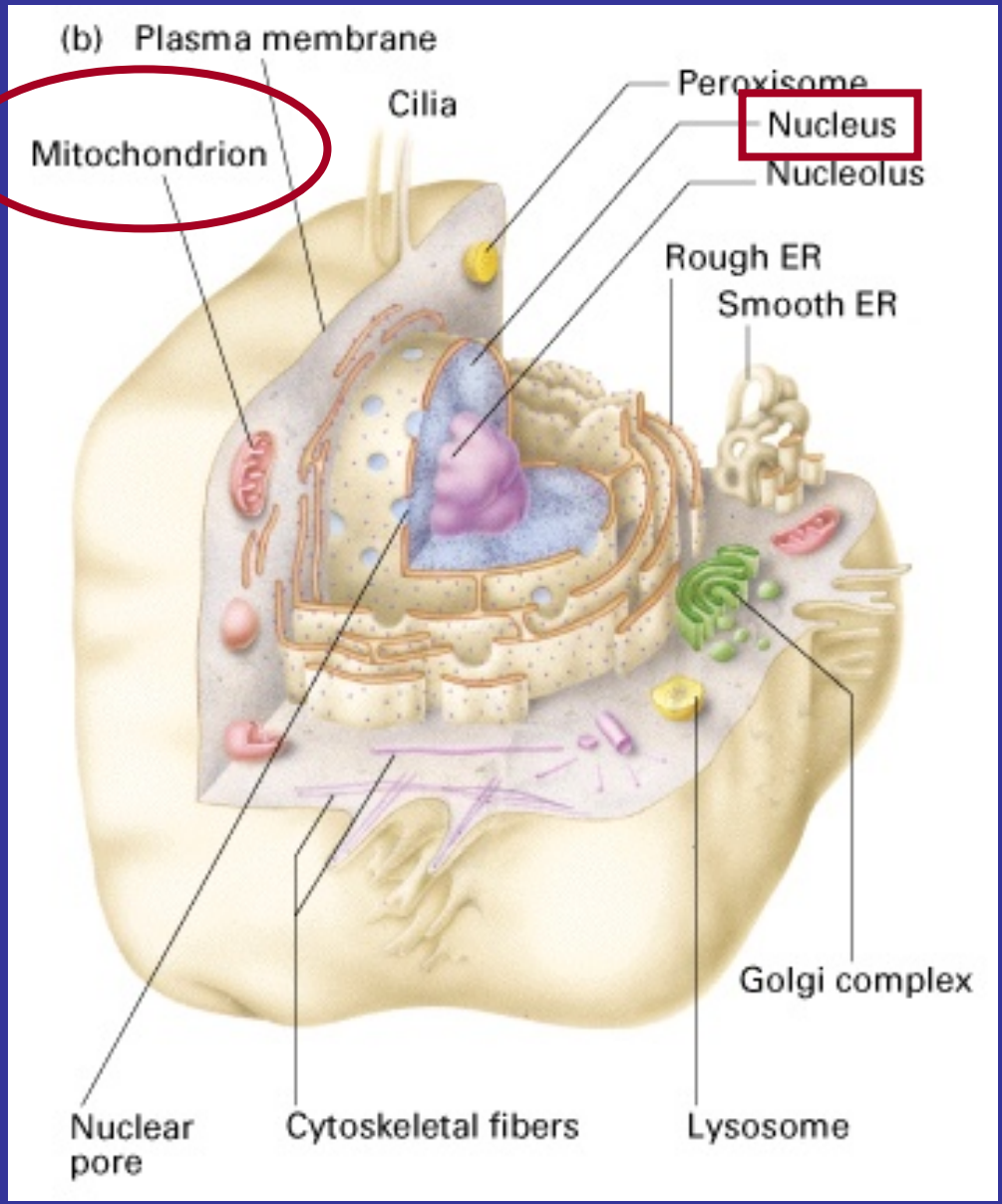
E. O. Wilson (2002)

Table 3.1. Historical overview of primary methods used to study genetic variation in natural populations.

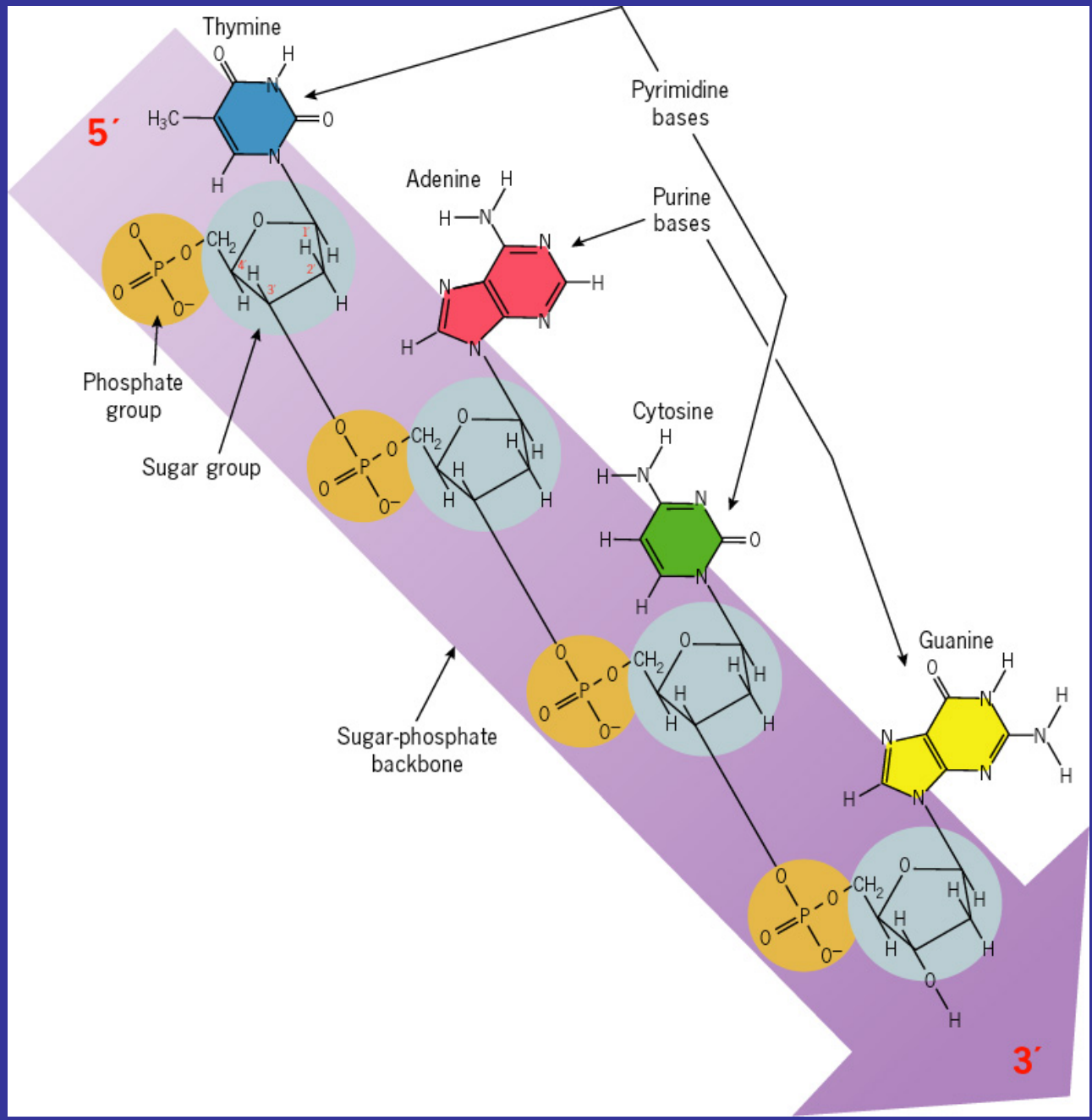
Time Period	Primary techniques
1900-1970	Laboratory matings and chromosomes
1970s	Protein electrophoresis (allozymes)
1980s	Mitochondrial DNA
1990s	Nuclear DNA
2000s	Genomics

DNA

~17,000 base pairs



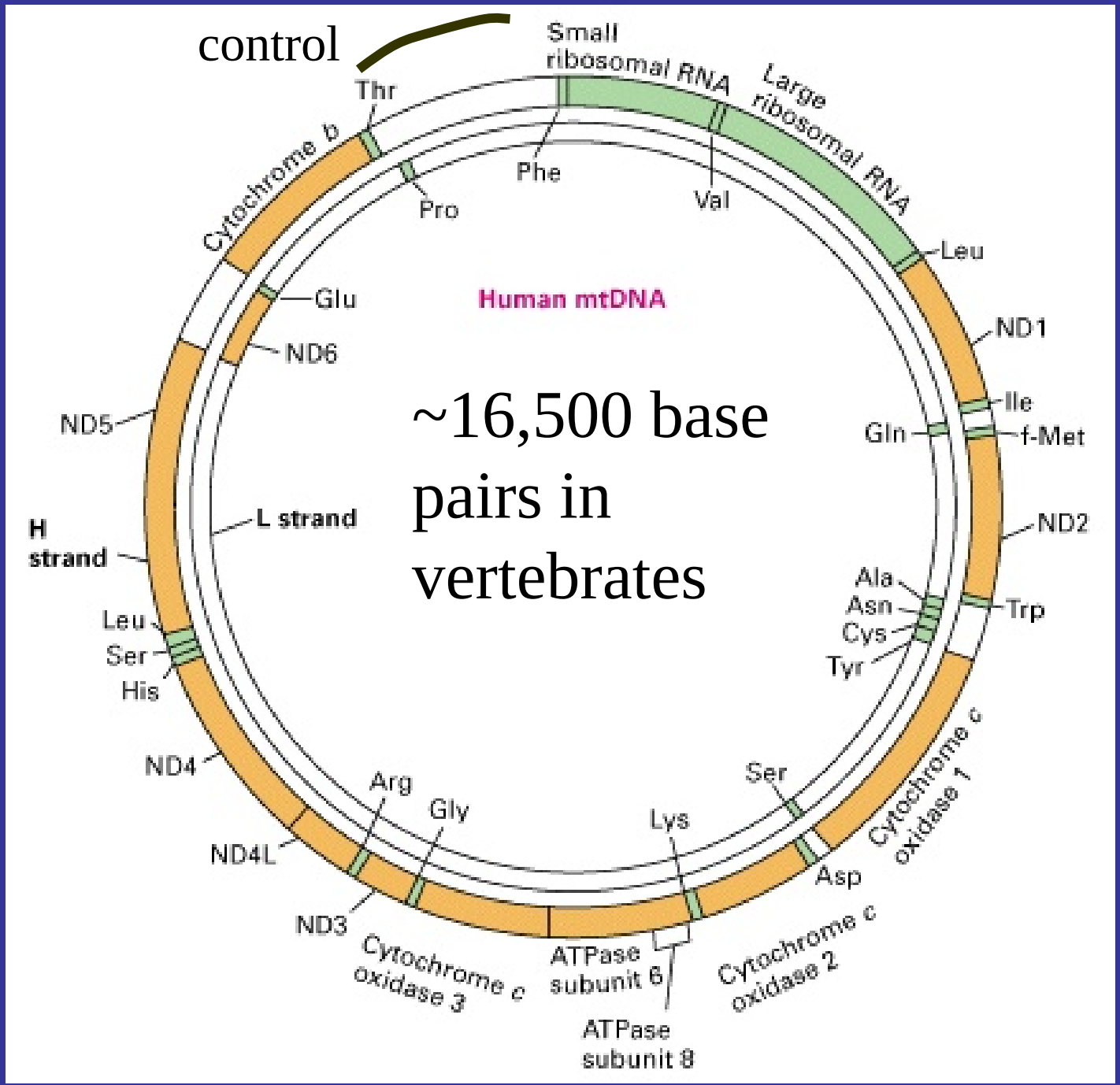
billions of base pairs


Thymine
5′
Pyrimidine bases
Adenine
Purine bases
Phosphate group
Sugar group
Cytosine
Guanine
Sugar-phosphate backbone
3′

Mitochondrial DNA (mtDNA)

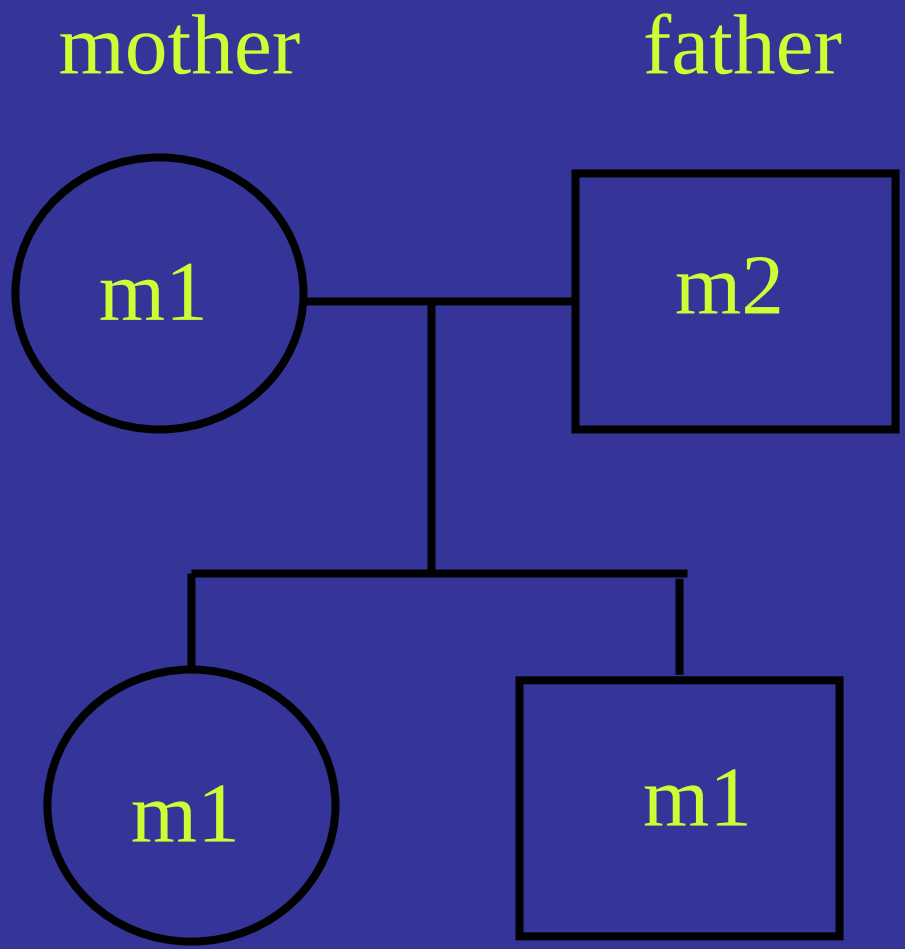
- Predominantly maternally inherited in plants and animals
- Haploid (one copy in each individual)
- No recombination
- Location of oxidative phosphorylation (OXPHOS), which is vital for metabolic activity of somatic cells and gametes
- mtDNA encodes 13 of 67 components of the OXPHOS system (54 nuclear)

mtDNA



~16,500 base pairs in vertebrates

Maternal inheritance of mtDNA



All children inherit their mother’s mtDNA type

Mitochondrial genome variation and the origin of modern humans

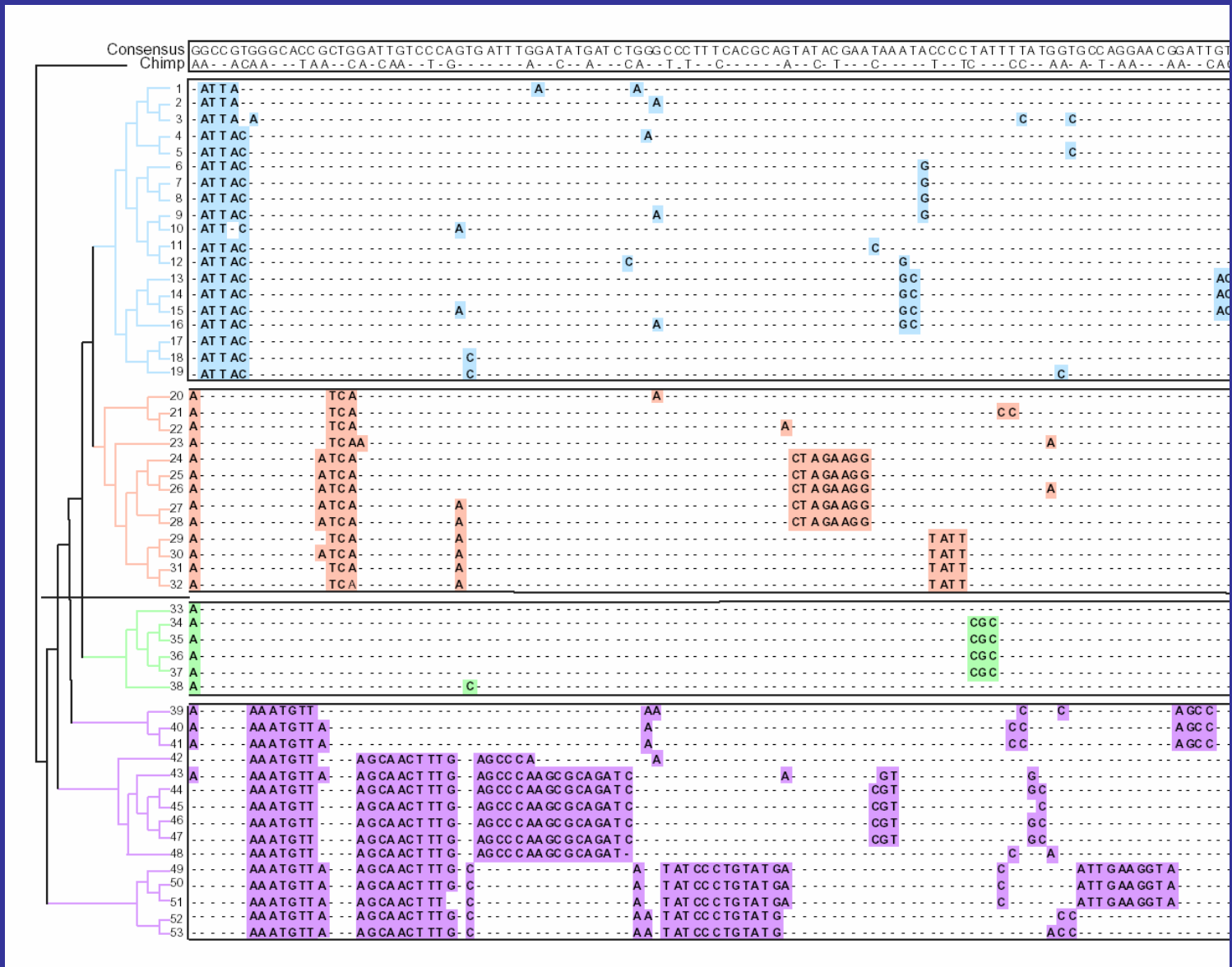
Max Ingman*, Henrik Kaessmann†, Svante Pääbo† & Ulf Gyllensten*

** Department of Genetics and Pathology, Section of Medical Genetics, Rudbeck Laboratory, University of Uppsala, S-751 85 Uppsala, Sweden*
† Max Planck Institute for Evolutionary Anthropology, Inselstrasse 22, D-04103 Leipzig, Germany

Compared 53 complete genomes (16,566 base pairs).

2000. Nature 408:708-713.

Within species polymorphism (humans)

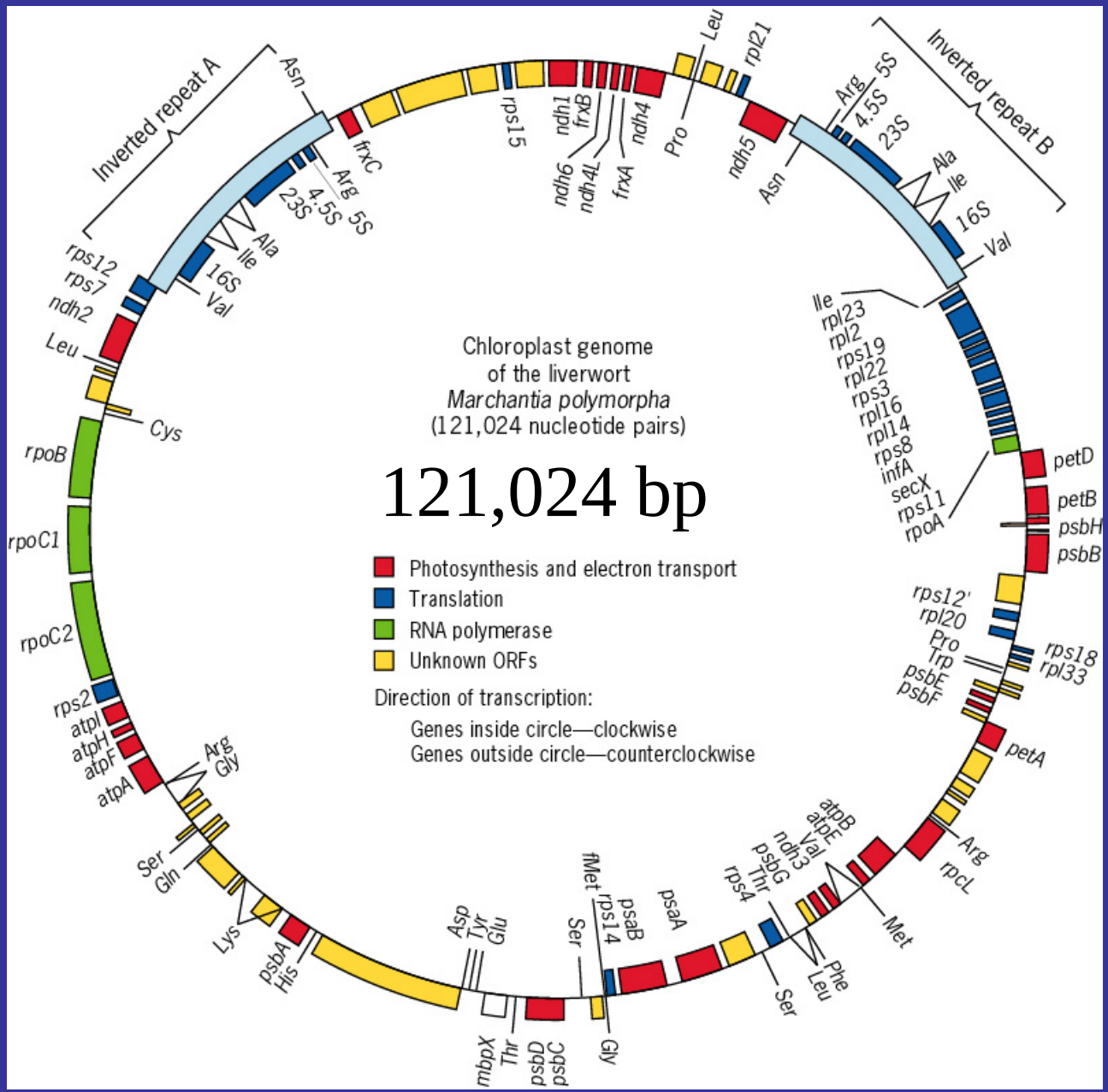


Asians & others

Asians

Africans

Chloroplast DNA



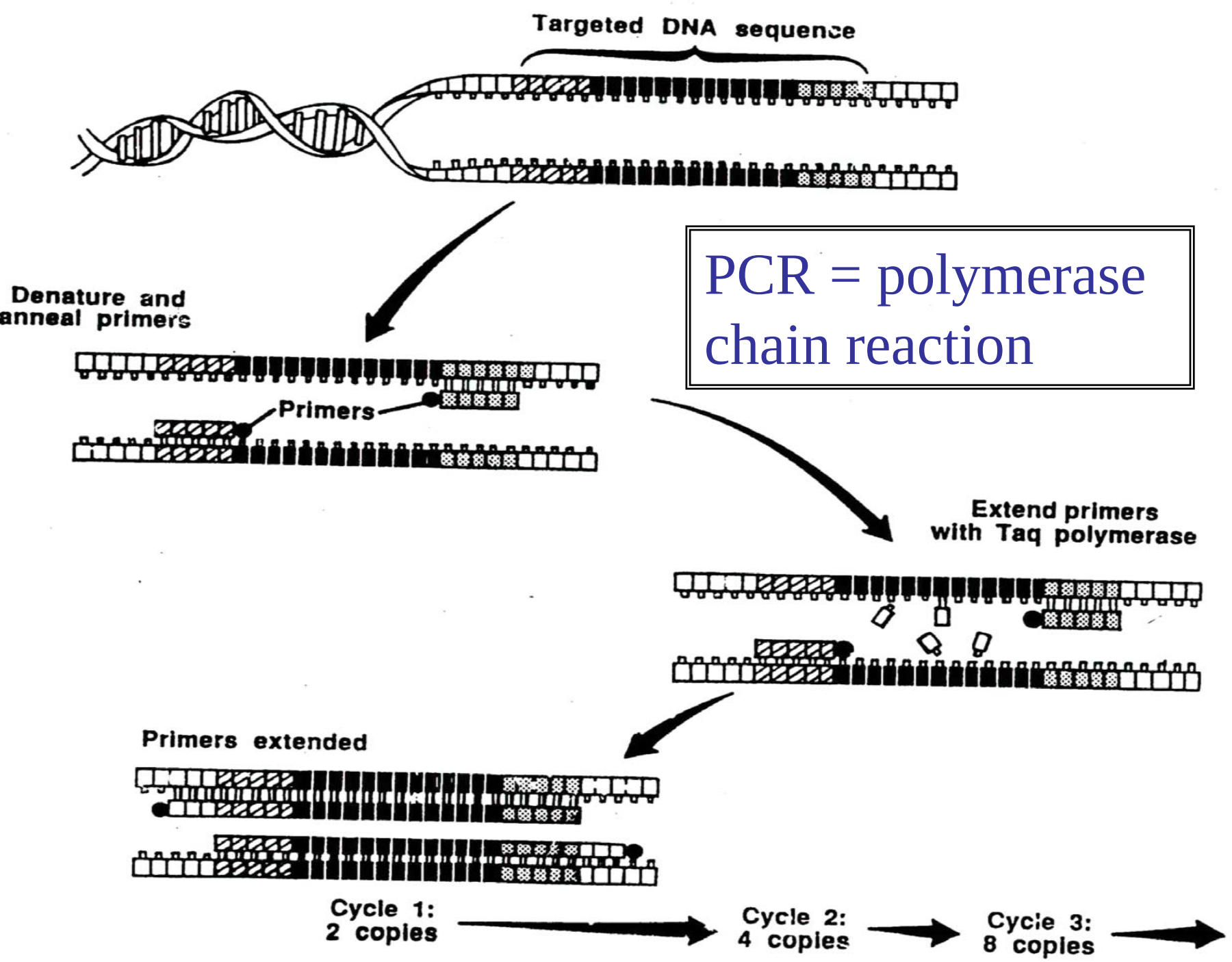
Liverwort
Marchantia polymorpha

Chloroplasts

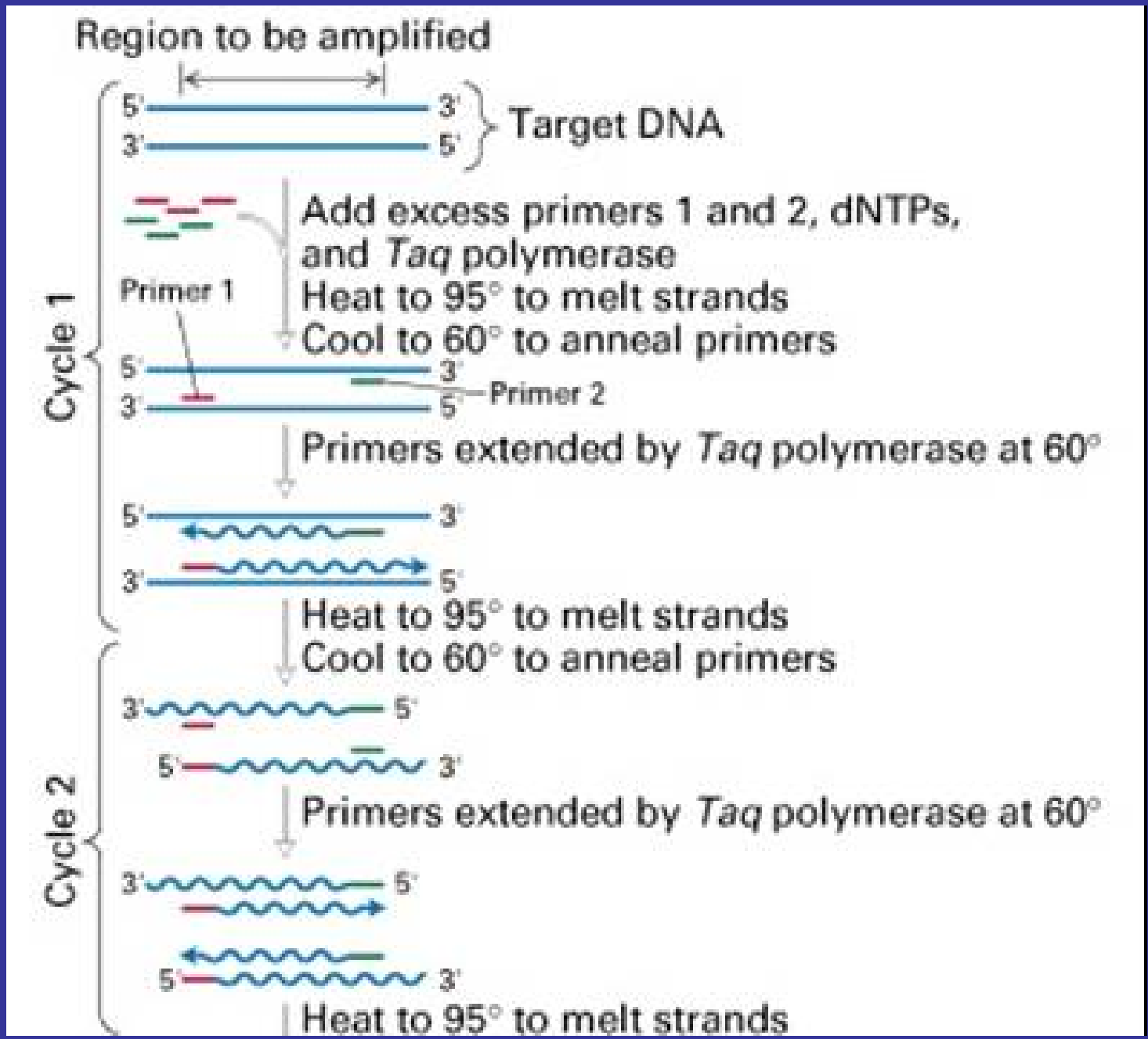
- Location of photosynthesis in plant cells
- Haploid (one copy in each individual)
- Maternally inherited in some groups and paternally inherited in others (pine trees)

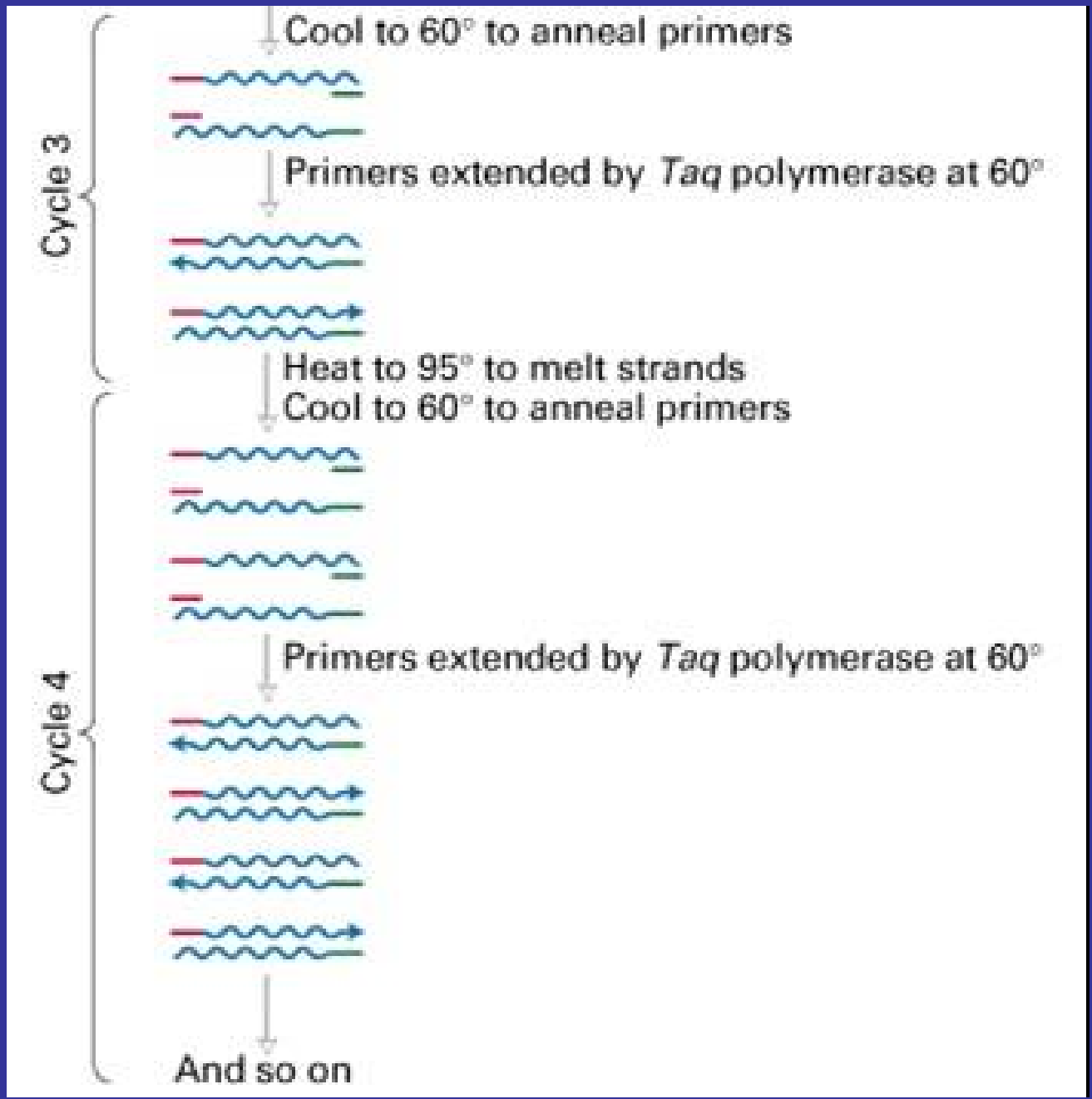
Polymerase Chain Reaction (PCR)

- Allows us to make many copies of a specific region of the genome (amplification)
- We need two primers that flank a certain gene or region in the species of interest
- Primers are short (~20 bp) synthetic strands of DNA that bind to DNA and start the replication process



PCR = polymerase chain reaction


Region to be amplified
5'
3'
3'
5'
Target DNA
Add excess primers 1 and 2, dNTPs,
and Taq polymerase
Heat to 95° to melt strands
Cool to 60° to anneal primers
Primer 1
Primer 2
Cycle 1
Primers extended by Taq polymerase at 60°
Heat to 95° to melt strands
Cool to 60° to anneal primers
Cycle 2
Primers extended by Taq polymerase at 60°
Heat to 95° to melt strands

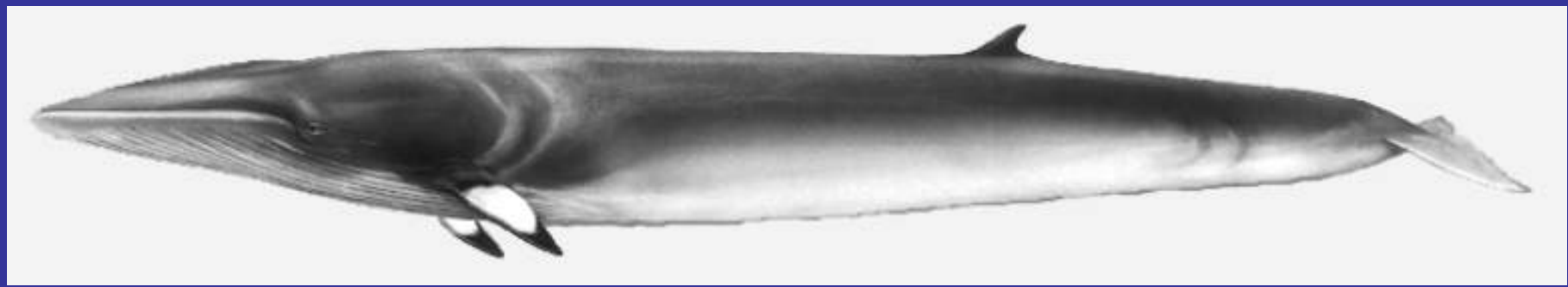

Cycle 3
Cool to 60° to anneal primers
Primers extended by *Taq* polymerase at 60°
Heat to 95° to melt strands
Cool to 60° to anneal primers
Cycle 4
Primers extended by *Taq* polymerase at 60°
And so on

Large spring near Great Fountain Geyser was the source of the culture of *Thermus aquaticus* used to make *Taq* polymerase. At the time of the discovery, its outflow was 70 degrees C (158 degrees F), the optimum temperature of *Thermus aquaticus*.

Thomas D. Brock
UW – Madison
1960's

Which Whales Are Hunted? A Molecular Genetic Approach to Monitoring Whaling

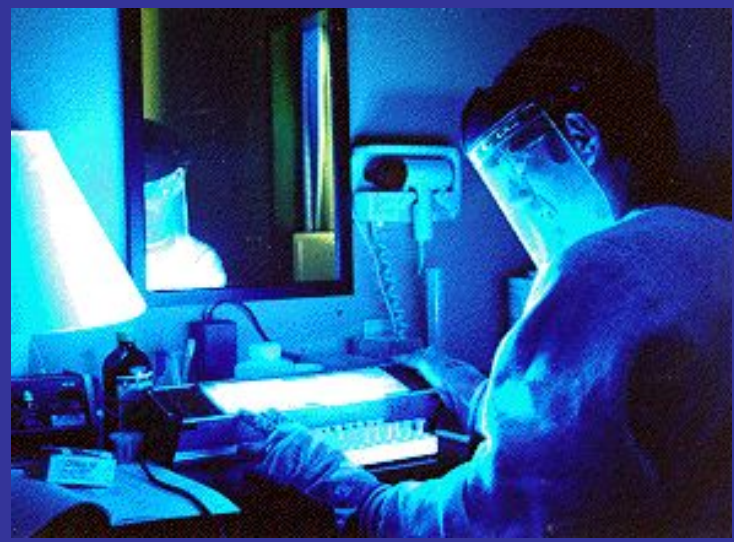
C. S. Baker and S. R. Palumbi



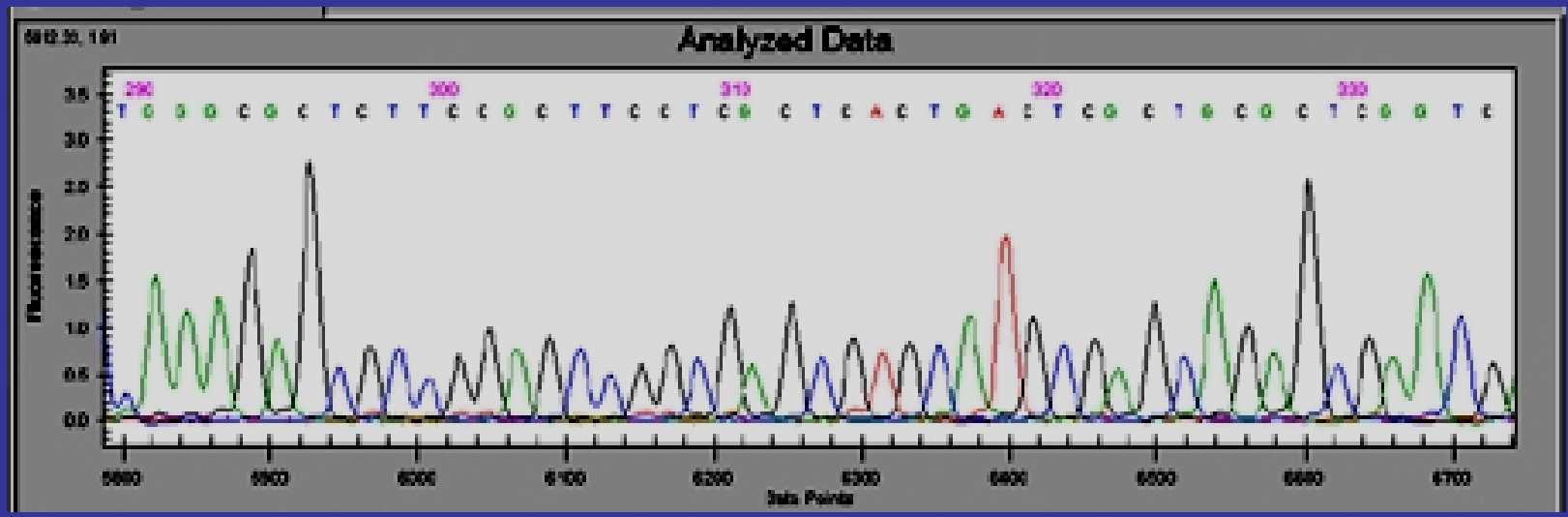
Minke whale

Tokyo fish market

Hotel PCR



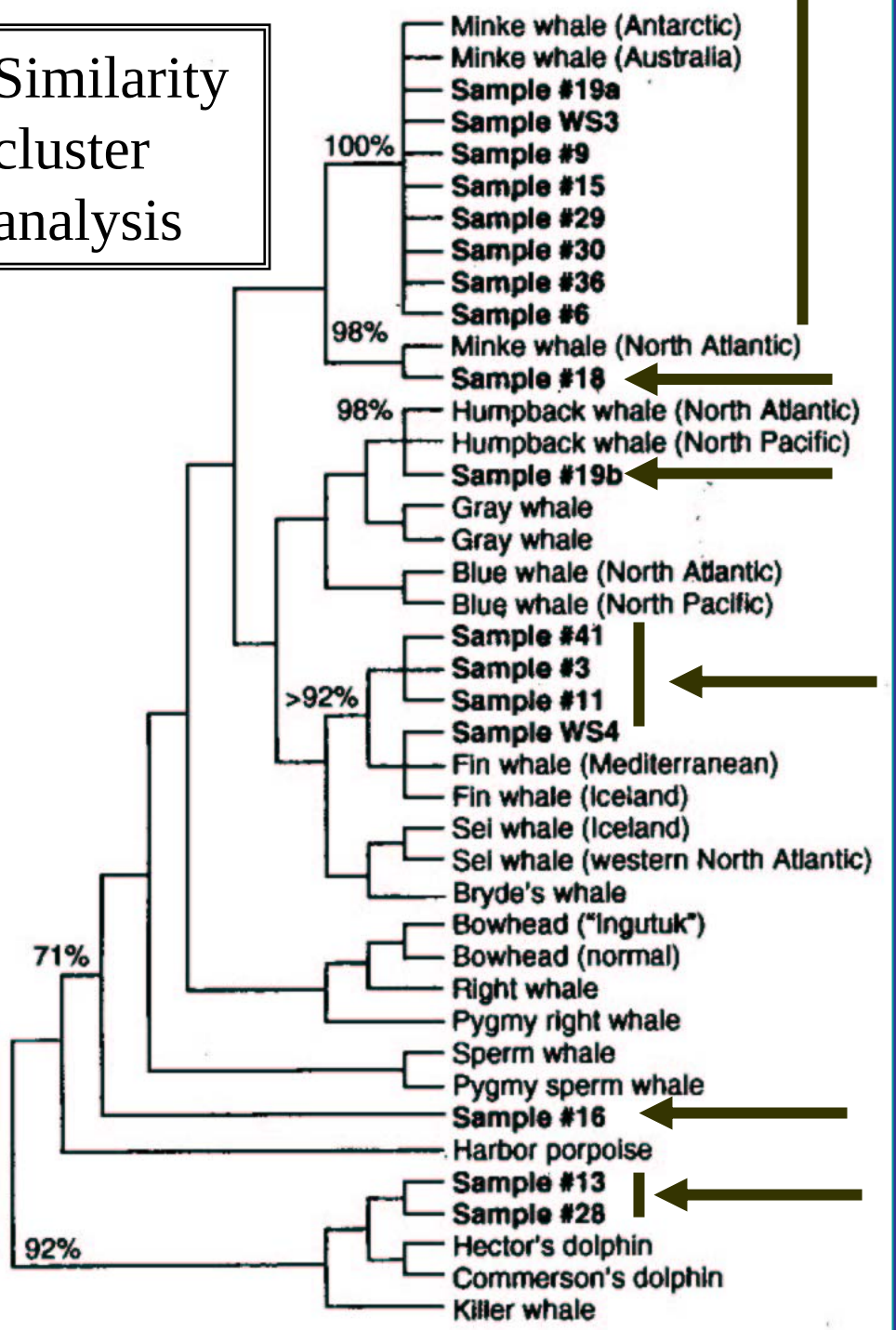
Mitochondrial DNA Sequencing



...CTGAAAGGGT... Haplotype A
...CTGGAAGGGT... Haplotype B

Base substitution

Similarity cluster analysis



Southern minke (8)

Northern minke

Humpback

Fin whale (4)

Unknown “whale”

Unknown “dolphins”(2)

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Andrea Taylor and friend

Microsatellite loci

or

VNTR (Variable Number of Tandem Repeat) loci

or

SSR (Simple sequence repeats)

or

STRPs (short random repeat polymorphisms)

Box 3.2. Microsatellite DNA sequence polymorphism.

Below is a locus on a chromosome showing two alleles from an individual heterozygous for a microsatellite dinucleotide repeat (AC; in bold and blue). Note the difference in allele length results from an addition of one repeat in the lower (paternal) allele. The primer-binding sites for PCR are underlined. They are highly conserved sites (usually ~20 bp long) flanking the highly variable microsatellite repeats.

Maternal chromosome (allele with 7 repeats):

primer site

5′ - TGACTTAGTGTATACATACACACACACACACTTATCGATCGAGCTAT - 3′
3′ - ACTGAATCACATATGTATGTGTGTGTGTGTGAATAGCTAGCTCGATA - 5′

primer site

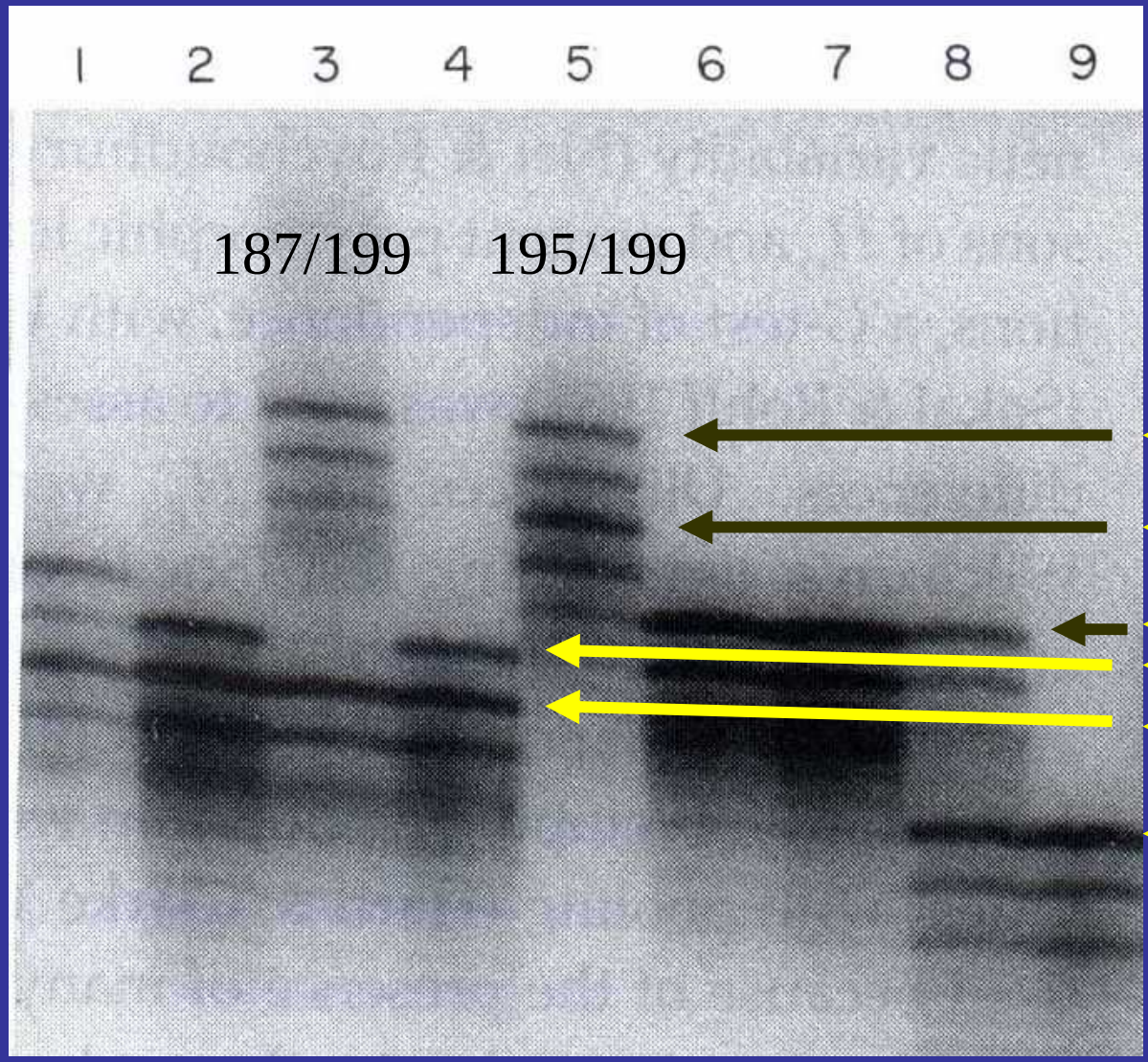
Paternal chromosome (allele with 8 repeats):

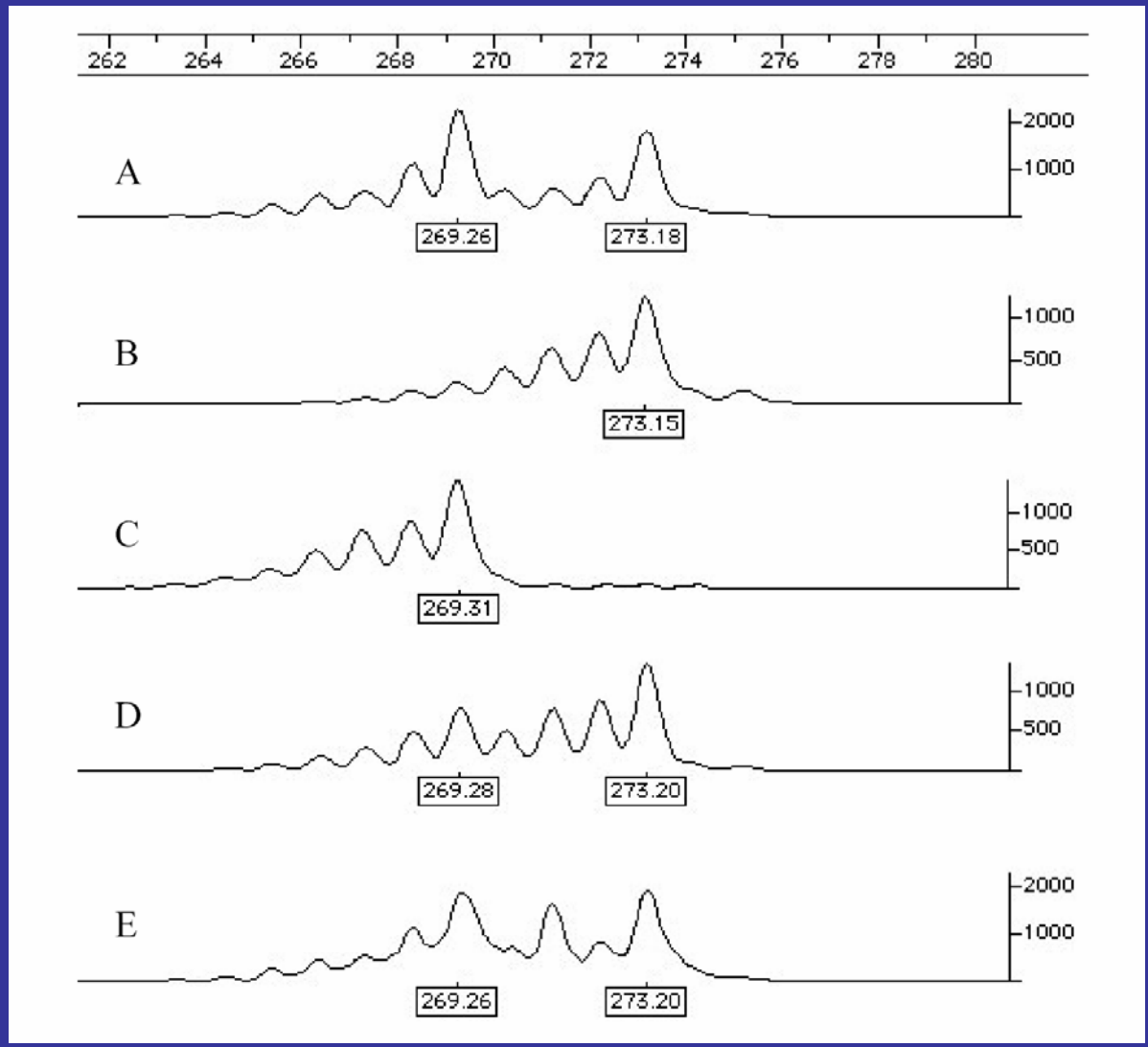
primer site

5′ - TGACTTAGTGTATACATACACACACACACACACTTATCGATCGAGCTAT - 3′
3′ - ACTGAATCACATATGTATGTGTGTGTGTGTGTGAATAGCTAGCTCGATA - 5′

primer site

Hairy-nosed wombat microsatellite





Genotypes

269/273

273/273

269/269

269/273

269/273 (?)

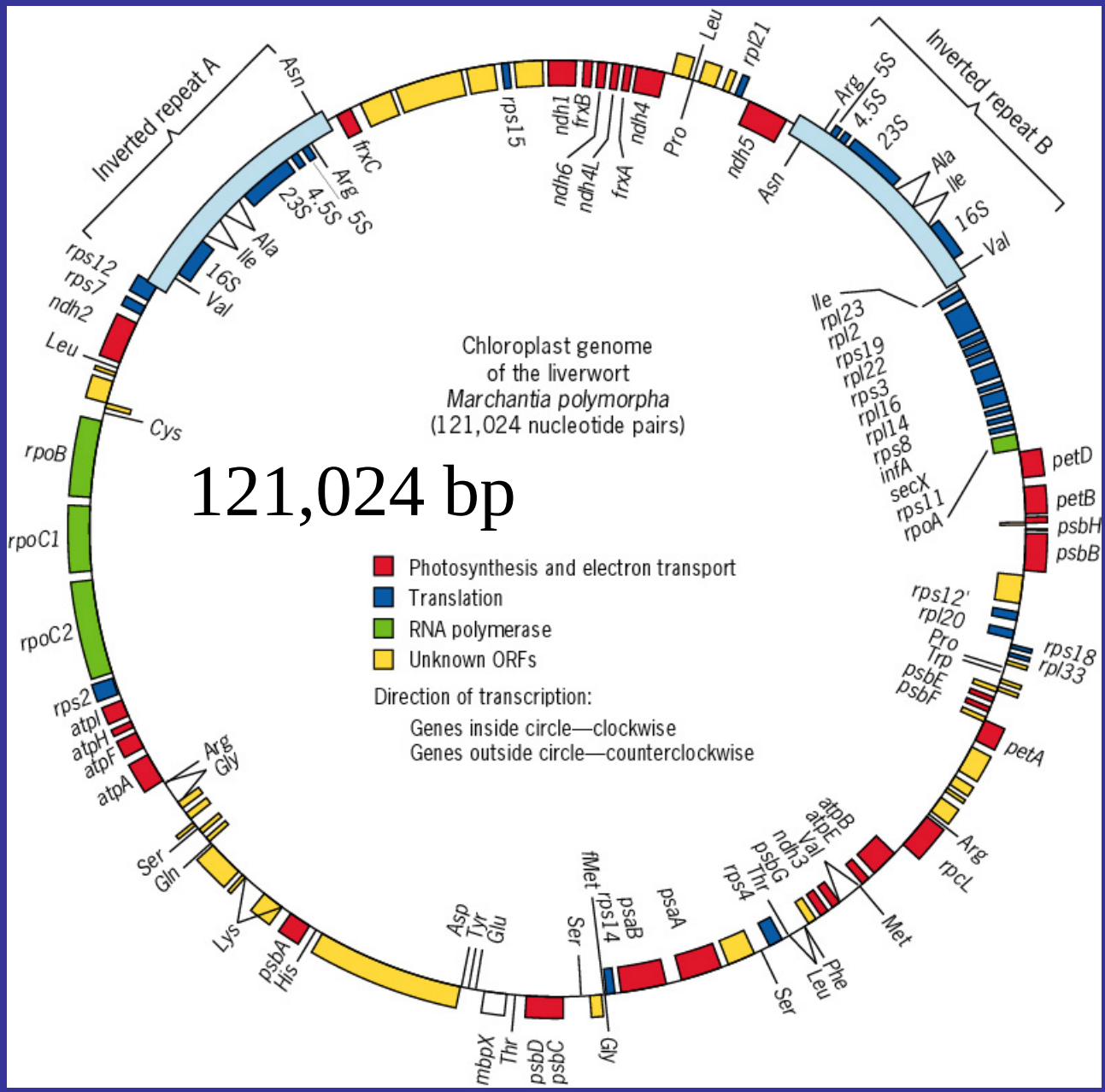
European Alpine ibex (*Capra ibex*)

Box 4.3. Modified GenBank sequence database entry for the *Lla71CA* locus in the hairy-nosed wombat (Figure 4.4). The primers in the sequence at the bottom have been capitalized and the repeat region (CA) is shown in bold. The n's in the sequence are basepairs that could not be resolved in the sequencing process.

```
1: AF185107. Lasiorhinus latif

LOCUS       AF185107                 310 bp    DNA     linear   MAM 01-JAN-2000
DEFINITION  Lasiorhinus latifrons microsatellite Lla71CA sequence.
  AUTHORS   Beheregeray,L.B., Sunnucks,P., Alpers,D.L. and Taylor,A.C.
  TITLE     Microsatellite loci for the hairy-nosed wombats (Lasiorhinus
            krefftii and Lasiorhinus latifrons)
  JOURNAL   Unpublished
  AUTHORS   Taylor,A.C.
    JOURNAL   Submitted (31-AUG-1999) Biological Sciences, Monash University,
            Wellington Rd., Clayton, VIC 3168, Australia
FEATURES             Location/Qualifiers
     source          1..310
     repeat region   109..154
                     /rpt_type=tandem
                     /rpt_unit=ca
BASE COUNT       99 a     94 c     42 g     68 t      7 others
ORIGIN
        1 gngctcggnn cccctggatc acagaatcta aatctgagca tctcagAATG AGAAGGTATC
       61 TCCAGGataa ccannnccct ctacctaaac aagaattcca ctcccctaca cacacacaca
      121 cacacacaca cacacacaca cacacacaca cacactcaat agacccaaca agtggaatgt
      181 cacacagcct ttggggnagg tgggggatat acttCCTATG ACATAGCCTA TACCacttct
      241 gaatagtaac tttcctatcc ataaatctaa aacctacttc ccactctttt ctgctagttc
      301 tataatctgg
```

Microsatellites in Chloroplast DNA!!!



Evolutionary Conservation of Ten Microsatellite Loci in Four Species of Felidae

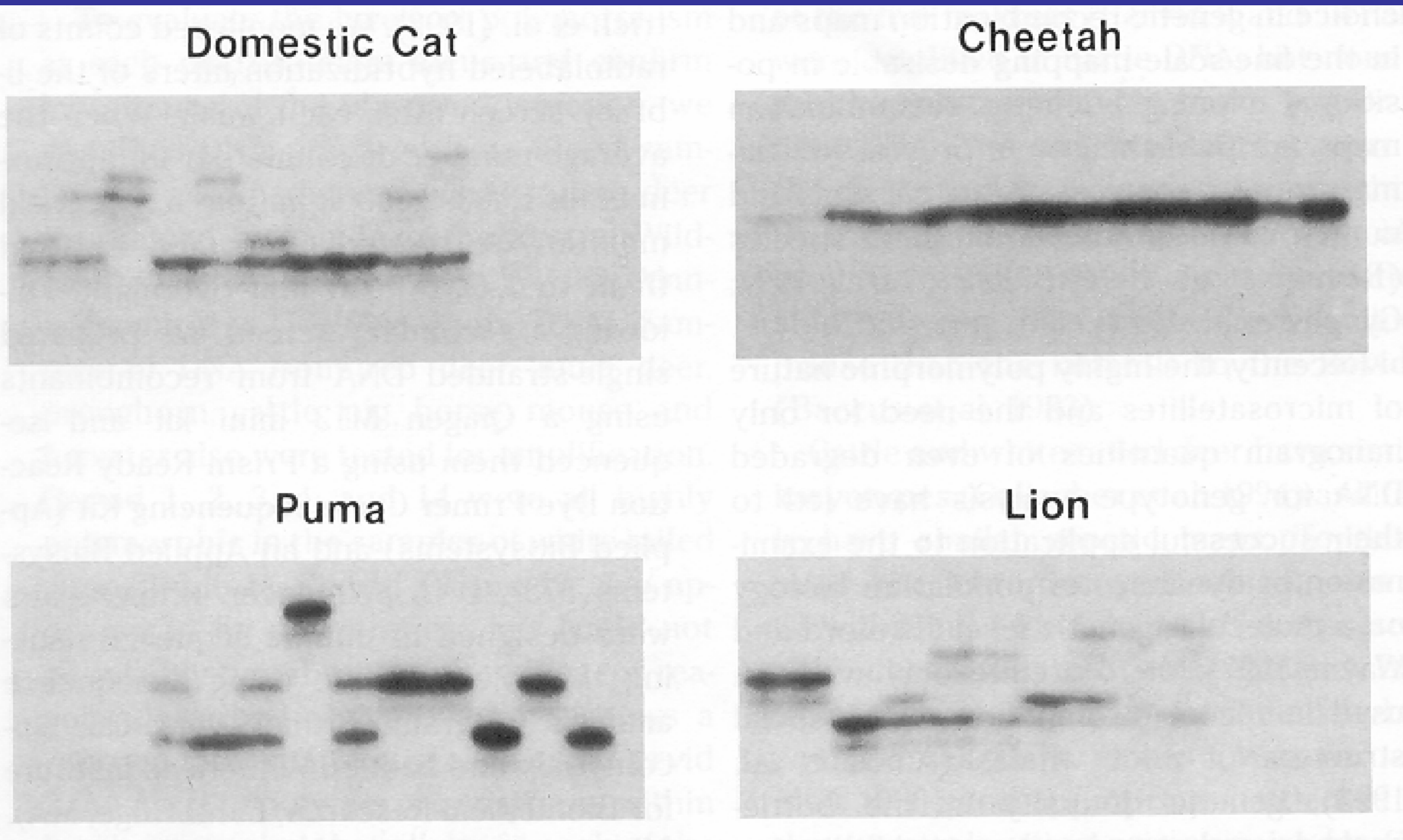
M. A. Menotti-Raymond and S. J. O'Brien

Journal of Heredity 86:319-322. 1995.

Heterologous microsatellite primers

Fca 8:	ACTGTAAATTTCTGAGCTGGCC
	TGACAGACTGTTCTGGGTATGG
Fca 23:	CAGTTCCTTTTTCTCAAGATTGC
	GCAACTCTTAATCAAGATTCCATT
Fca 35:	CTTGCCTCTGAAAAATGTAAAATG
	AAACGTAGGTGGGGTTAGTGG
Fca 43:	GAGCCACCCTAGCACATATACC
	AGACGGGATTGCATGAAAAG
Fca 45:	TGAAGAAAAGAATCAGGCTGTG
	GTATGAGCATCTCTGTGTTCGTG

Domestic cat primers



Fca77

Is there mtDNA sequence information for your favorite species?

GenBank

National Center for Biotechnology Information (NCBI)

Individual ID

Through the most sensitive DNA testing, RFLP testing, the FBI Laboratory determined conclusively that the semen on Ms. Lewinsky's dress was, in fact, the President's. The chance that the semen is not the President's is one in 7.87 trillion.

Prob = 1 / (7,870,000,000)

Microsatellites as genetic tags

(mark-recapture)

Dry Fork Valley

Two Medicine River

Rub tree

Ancient DNA

Molecular Ecology 1997, 6, 487–492

SHORT COMMUNICATION

Analysis of microsatellite DNA from old scale samples of Atlantic salmon *Salmo salar*: a comparison of genetic composition over 60 years

E.E. NIELSEN, M.M.HANSEN† and V. LOESCHCKE
Department of Ecology and Genetics, University of Aarhus, Ny Munkegade, Building 540, 8000 Aarhus C, Denmark
†Danish Institute for Fisheries Research, Department of Inland Fisheries, Vejlsøvej 39, 8600 Silkeborg, Denmark

Abstract

Microsatellite analysis was applied to scale samples of Atlantic salmon collected up to 60 years ago. Samples from the 1930s, from a now endangered Danish population, were compared with recent samples (1989), to test if the present population consists of descendants from the original one. Allele frequencies had changed over time, but individuals from the two samples caught about 60 years apart clustered together when compared with the closest neighbouring population and another reference population. However, fewer alleles were detected in the recent sample from the endangered population, most likely due to a population bottleneck or sampling artefacts.

Locus	Allele	Skjern River 1989	Skjern River 1930s	Conon	Ätran
SSOSL 85	177		0.007		
	179		0.010		
	181		0.007		
	183	0.11	0.316	0.08	0.15
	187		0.003		
	189		0.003	0.46	0.14
	191		0.003	0.02	0.02
	193	0.06	0.076	0.08	0.25
	195	0.40	0.330	0.02	0.02
	197		0.017	0.04	0.13
	199	0.28	0.073	0.21	0.11
	201	0.14	0.142	0.02	0.06
	203			0.08	0.04
	209				0.02
	217		0.003		0.04
	221	0.01	0.007		0.02